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SIGNIFICANCE OF BACTERIAL INFECTION IN HOG CHOLERA

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THE SIGNIFICANCE OF BACTERIAL INFECTION IN HOG CHOLERA

R. R. DINWIDDIE

The view now generally held by pathologists who have interested themselves in this disease of hogs is that pure uncomplicated hog cholera is itself a relatively mild disease which derives its malignancy from the bacterial infections which follow in the wake of the primary infection with the true virus of the disease.

In this view cholera of hogs would, in its tendency to complications, be comparable to influenza of man.

Of the bacterial infections which accompany hog cholera the most attention has been given to two species *B. suipestifer* and *B. suisèpticus* each of which have been regarded as disease germs capable of begetting independant diseases in hogs.

Although this view is no longer generally entertained in regard to the first named of these organisms *Bacillus suipestifer* (*B. cholerea suis swine pest or hog cholera bacillus*) there is still much obscurity or, at least, lack of positive knowledge, as to the significance of its occurrence in hog cholera and its influence on the course of the disease in individual animals and in cholera outbreaks as a whole.

In the preparation of antiserum, also, and in the handling of outbreaks of swine infections by serum immunization it is obviously of importance to know in what degree, if any, the results of vaccination may be influenced by the presence or absence in the serum of antibodies to bacterial infection.

In the case of *Bacillus suisepticus* the significance of its association with the essential virus of hog cholera is in one way no less uncertain and also no less important since here the question of an independant infection with this germ and consequent infectious disease of hogs unassociated with cholera has to be considered. The disease which was differentiated from hog cholera by Th. Smith in 1891 and called by him "Swine Plague" from its correspondence in pathology and etiology to the Schweineseuche of the Germans is still included by veterinary writers among the hog epizootics or enzootics of this country. There is no positive evidence that such a disease exists in the United States and, in fact, no published data upon which an opinion could be based other than that of Smith referred to above. In the light of more recent discoveries in the etiology of hog cholera it is a question whether swine plague outbreaks can be regarded other than as complications of true hog cholera infection.

Mention merely of the bacillar swine pest (*B.Suipestifer, Voldagsen*) *of Dammaun and Stedefeder** and of the "Typhus and paratyphus des Schweines" of Glasser† will show the variety of bacterial infections which have yet to be

*Jahresberich Vet. Med. Jahr-1910.

†Krankheiten des Schweines.

definitely established as independant diseases or shown to be complications of true hog cholera infection.

In the scientific investigation of contagious diseases of swine and even more for practical purposes of prophylaxis by antiserum vaccination the chief difficulty is the lack of any biologic or laboratory method of exact diagnosis of true hog cholera infection.

An antigen-antibody reaction which has been the object of prolonged research in this laboratory has not yet been found although not yet abandoned as hopeless. Agglutination tests of *B. suipestifer* cultures with serum of cholera diseased hogs is without value in diagnosis. I would emphasize this fact since assertions to the contrary have been frequently made.

A finding of this bacillus disseminated through the body and recoverable by the usual cultural technique may be diagnostic of hog cholera but this could hardly be assumed while the significance of such infections is under investigation. We are, therefore, limited in diagnosis to the history, symptoms, post mortem findings and inoculation tests with filtered virus, the latter a cumbersome and not always reliable procedure.

With the variety of infectious swine diseases which are described in European veterinary literature it is scarcely conceivable that all the outbreaks in this country could be due to cholera infection. In view of the extraordinary demand for serum vaccination which has sprung up in recent years and with it the equally extraordinary dissemination of hog diseases throughout the country there seems to be an urgent need of more extensive and more thorough field investigations in order that the application of this means of controlling hog diseases be put on a rational and scientific basis.

Bacillus suipestifer in hog cholera. When bacteriologic studies are made of the organs of hogs dead of cholera, the material being obtained in good condition, one obtains by the usual technique results which may be classified thus:

1. Culture media remains sterile and miscroscopical study of stained films shows the absence of bacteria. The result is entirely negative.
2. Culture tests negative, stained films positive. Bacteria are present which fail to grow in culture tubes.
3. Culture tests positive, stained films negative. The bacteria present are so scarce that they are not found on microscopical examination.
4. Positive results to both culture tests and microscopical study.

Wherever positive results are obtained to culture tests the organism present will be found in the great majority of cases to correspond to that described as *B. suipestifer (B. cholerae suis)*. So frequently is this species found disseminated in the blood and organs in hog cholera that there is a natural reluctance to abandoning the view that it is possessed of primary etiologic significance. A satisfactory explanation of the apparently symbiotic relation between the filterable virus of cholera and this bacterial species has not yet been advanced nor is there unanimity of opinion as to the influence of this mixed infection on the gravity of the disease or on the post-mortem lesions found. The view that is generally held, namely, that this germ is a constant inhabitant of the intestinal canal in hogs which finds oportunity to invade the tissues and system only when a prior infection with the true hog cholera virus occurs, obtains some support from the discovery of organisms corresponding to *B. suipestifer* in a certain percentage of normal or non-cholera in-

fected hogs. That certain animals in the same outbreak may show this bacillar complication and others not, could be explained by the relative abundance or scarcity of the germ in the intestine or by the intensity or localization of the primary infection. Similarly might be explained the absence of this complication in all the animals of any outbreak.

The influence of contagion—transference of the bacillar infection—in determining the occurrence of this organism in cholera infected animals is a matter about which nothing is known.

Bearing upon this question also is the fact that such a mixed infection may occur after inoculation of filtered virus which apparently contained no bacilli (and in which the inoculated animals were isolated from other infected hogs). (See the filtration experiments of Dorset, Bolton and McBryde). In such it is difficult to conceive of any but an endogenous source for these germs.

That the local intestinal lesions in the form of continuous or isolated patches of exudate, superficial erosions, or thick based ulcers are due in any specific manner to the action of *B. suipestifer* does not admit of proof, although strongly suggested by the results of feeding experiments with cultures of this germ. Other organisms, however, may be concerned in producing these local changes or even in initiating them.

We have been able by inoculation methods to show the presence of virulent strains of *B. suisepticus* at the base of typical hog cholera ulcers and at the same time (by the use of rabbits immunized against this species) to show the apparent absence of virulent hog cholera bacilli. *

In order to learn something of the effects attributable to mixed infection and the influence of contagion (bacillar) in producing this condition post mortem examination and bacteriologic culture tests have been made as far as possible on all material available during the past two years. This includes experimentally infected animals, pigs artificially infected for serum production, hogs condemned for cholera at the packing plant or city abbattoir at Little Rock and material obtained from outbreaks of hog disease on farms in this State.

In respect to the influence of contagion in begetting mixed infection the data is necessarily incomplete except in the case of experimental stock. Virus pigs, that is, those purposely infected in order to obtain virulent blood for immunizing purposes, were generally both inoculated and exposed in pens infected with mixed strains of virus. Much of the material from all sources (except experimental stock) was, on account of post mortem change, unsuitable for bacteriologic study and the records of such are not included in the table.

The bacteriologic tests applied in this investigation were made for the purpose of determining the presence of systemic infection,—dissemination of bacteria in the blood or internal organs. For this purpose the usual technique was employed. Pieces of tissue ordinarily of the volume of bird shot or larger were transferred regularly from spleen and liver, occasionally also from kidney and lung to fluid and solid culture media in plain tubes and in fermentation tubes. Only in a few instances were culture tests made of blood from the heart. Culture tubes were incubated at 36° to 38°C. and retained for six days when no growth became visible. The intent was mainly to discover the conditions under which bacilli of the "hog cholera bacillus" type occurred

*Ark. Exp. Station Bul. No. 105. (1910).

but studies were made of certain other organisms which appeared in culture tubes or on stained films from the organs with more or less frequency. A *growth in dextrose broth fermentation tubes with development of 25 to* 40 per cent volume of gas and in lactose broth tubes without evolution of gas, the growth in plain nutrient broth tubes showing moderate cloudiness in 24 hours and consisting of motile organisms with the morphology of *B. suipestifer* was considered presumptive evidence of the presence of that germ.

Out of the large number of cultures fulfilling these requirements seven, which seemed to show slight variation from the type in gas production or morphology on preliminary examination were selected for more complete study and comparison made especially with reference to their fermentative *action on sugars, agglutination with antiserums and pathogenicity for* laboratory animals. Only one of these strains was tested as to its virulence for hogs.

Besides the cultural tests referred to, direct inoculation of diseased organs of hogs to guinea pigs was made use of in a small number of cases. By this means one can isolate the *bacillus suisepticus* (when the strain is virulent) from pneumonic lesions showing a mixture of species more readily than by plate cultures.

In regard to the material from the serum plant it should be noted that this differs in two important particulars from that obtained from farm outbreaks or experimental pens. These animals (listed as "virus pigs") were killed often several days before death would naturally have occurred: they were also usually infected by strains of cholera virus of which no bacteriologic study had been made. From these, of course, no data is available as to the influence of contagion (or transmission by inoculation) in producing bacterial infection.

As to the lesions tabulated, the most prominent in each case are printed in itallics. Splenic engorgement indicated by the word "spleen" was frequently only of slight degree and occasionally doubtful; "Lymph nodes" refers to deep congestion or blood shot areas in cortex: "hemorrhages" to punctiform or larger ecchymoses on serous or mucous membranes. Pneumonia is only listed when the area involved is of large extent.

Table I. BACTERIAL INFECTION (GENERALIZED) IN FARM OUTBREAKS.

No.	Stage of disease	Bacterial Findings	Lesions	Remarks
1	Moribund	Negative	No plain lesions found	*Infected at fair*
2	Killed	Negative	Pneumonia	Same farm as No. 1
3	Moribund	Cocci	*Spleen*; kidneys; lymph nodes; pneumonia	Same farm as No. 1
4	Died	Streptococci	*Spleen*; lymph nodes; kidney	Acute disease
5	Died	Filamentous bacilli cocci	*Gastric*; spleen; ulcers	Only case on farm
6	Died	B. suipestifer	Spleen; *lungs*; large intestine deeply inflamed	Several others sick
7	Died	Negative	*Lungs*; (purulent pneumonia); cecal mucous membrane inflamed	Same farm as No. 6
8	Died	B. suipestifer. (B. pyocyaneous from lung)	*Lungs*; spleen; l. nodes; ulcers	Same farm as No. 6
9	Died	B. suipestifer	*Lungs*; kidney; spleen	Same farm as No. 6. (small pig).
10	Died	B. suipestifer	Spleen; l. nodes; pneumonia; pleurisy	Same farm as No. 6. (small pig).
11	Died	Negative	Pneumonia; pericarditis	Same farm as No. 6
12	Died	Negative	Lymph nodes	
13	Killed	Negative	Spleen; l. nodes; sloughs in colon	Same farm as No. 12
14	Killed	Negative	*Pericarditis*; pleurisy; pneumonia; *sloughs in cecum (slight)*	Three others died; all the *hogs on farm*
15	Died	Negative	Spleen; erosions in cecum	Large fat hog
16	Died	Negative	Kidney; l. nodes	Large fat hog; same farm as No. 15
17	Killed	Negative	*Skin*; kidney; *cecal ulcers*	City abbatoir
18	Killed	Negative	*Skin*; lymph nodes	City abbatoir
19	Killed	Negative	*Lymph nodes*; spleen; hemorrhages	Packing house
20	Killed	Negative	*Lymph nodes*; spleen; hemorrhages	Packing house
21	Killed	Negative	*Skin*; spleen; **kidneys**	*City abbatoir*

Table I. BACTERIAL INFECTION (GENERALIZED) IN FARM OUTBREAKS—*Continued*

No.	Stage of disease	Bacterial Findings	Lesions	Remarks
22	Died	Negative to culture tests; anerobic bacilli numerous	Gastric; lymph nodes; spleen and liver gassy	Diagnosis doubtful
23	Died	Negative	Spleen; kidneys	
24	Killed, 10th day	Large sporulating bac	Spleen; kidneys; ulcers (slight)	Same lot as No. 23
25	Killed, 10th day	Negative	Kidneys; spleen (slight lesions)	Same lot as No. 23
26	Killed, 10th day	Negative	Lungs; hemorrhages in colon	
27	Killed, 10th day	Spleen negative; liver B. Coli	L. nodes; *sloughs in colon*	*Same farm as No. 26*
28	Died	Spleen negative; liver non-fermenting *species*	Pneumonia; pleurisy; ulcers	Same farm as No. 26
29	Died (sick 10 days)	Cocci and non-fermenting bacilli	*Pneumonia; pericarditis;* spleen; kidneys; slough in cecum	Chronic case. Same farm as No. 26
30	Killed	Negative	L. nodes; sloughs in colon	Nos. 30 to 33 inclusive on same farm
31	Died	Negative	Gastric; lungs; ulcers in colon	
32	Died	Negative	Skin; spleen; l. nodes; ulcers	
33	Moribund	Negative	L. nodes; peritonitis; pericarditis; pleurisy; spleen; ulcers (slight)	
34	Died	Negative	Spleen; skin; sloughs in colon *(slight)*	Nos. 34 to 37 inclusive were *on same farm*
35	Moribund	Negative	Lymph nodes; ulcers	
36	Killed	Negative	Lymph nodes; ulcers (slight)	
37	Killed	Negative	Lymph nodes; ulcers; spleen	
38	Killed	Negative	Lymph nodes; spleen; hemorrhages	
39	Died	Negative	Kidneys; cecal erosions	Specimens brought to laboratory
40	Died	Negative	Spleen; sloughs in colon	Same farm as No. 39

In the 40 cases listed in Table 1 all of which with one possible exception were cholera infections, *B. suipestifer* was found generalized in four, the results were negative in thirty (that is, no growths obtained), streptococci were present in one, B. Coli (from liver only) in one, cocci in three, and unidentified nonfermenting bacilli in three, these latter representing three different species. In two of the cases in which *B. suipestifer* was generalized there were no gross intestinal lesions. Splenic engorgement, though present in all four, was not excessive in any. Pneumonia of large extent was also a constant condition in these four. In none of those in which cutaneous lesions are recorded was there a generalized bacterial infection.

The experimental work carried out with this material was (1) for purposes of diagnosis in doubtful cases and (2) to learn if there was any uniformity in the bacterial findings in the original material and in animals purposely infected by it, in other words, if the bacterial mixed infection in hog cholera is itself a result of transmission from animal to animal.

Cases listed as 1, 2 and 3 in Table 1 were from a small bunch of pure bred hogs, large and small, exhibited at a fair where they received infection and carried the disease back to the farm. Serum vaccination had little effect in curtailing the losses, probably from being undertaken too late.

In the first two cases, although the animals were very sick when killed, there were no post-mortem lesions by which a diagnosis of cholera could be made. In the third the pneumonia, involving the greater part of both lungs, was the prominent symptom although there was also splenic engorgement and hemorrhagic lymph nodes with a few petechiæ on kidney. There were no intestinal lesions. Culture tests were negative for bacilli of the hog cholera group. A coccus resembling morphologically *M. Catarrhalis* was obtained from the spleen. Preparations from the lung showed microscopically numerous organisms of varied species including small bi-polar staining ovals.

With the blood of this hog (No. 3 of Table 1) the followng inoculation tests were made:

EXPERIMENT I. One susceptible pig weighing 60 lbs., and one smaller pig 2 months old which was immune to cholera (one of a litter from a hyperimmune sow all of which were found to be immune at that time) received 4cc in muscles of thigh. They were kept together in a disinfected shed.

Result. The larger pig (susceptible) became sick on the sixth day and was killed when near to death on the tenth day. It showed cholera lesions without pneumonia (engorged spleen, inflammation with adherent exudate on gastric mucosa, and a similar condition in cecum and colon.)

Culture tests from liver and spleen were negative but bacilli of the colon group were present in the bile.

The other pig (immune to cholera) showed no symptoms of sickness.

EXPERIMENT II. From the blood of the diseased animal of the first experiment a second inoculation test was made. A susceptible 60 lb. pig and two immune pigs as controls were inoculated each with 6cc of unfiltered blood.

Result. The two latter remained unaffected. The other was obviously sick only after twelve days but by the sixteenth day was in a dying condition. It showed slight discoloration of the skin, spleen large, dark and soft, engorged lymph nodes, petechiæ on kidney and hemorrhages of large extent in mucosa of colon. There were no pectoral lesions.

Culture tests were negative.

In these experiments it is shown that a disease showing the characters of swine plague rather than of cholera, and which serum vaccination had failed to control, gave rise to fairly typical cases of cholera in inoculated animals without reproducing the pectoral lesions. The bacterial species (Coccus) present in organs of the original animal was not found in those of the animals infected from it. Bacilli of the hog cholera type were not found in any of the series

EXPERIMENT III. In order to learn whether this strain of virus would uniformly reproduce itself without this common bacterial mixed infection a further inoculation test was made with the blood of the last mentioned hog Large doses were employed with the idea of begetting a more acute disease.

Tw o susceptible pigs weighing about fifty pounds each received each 10cc. in muscles of thigh. They were kept together in a dissinfected shed.

Sickness was evident in both on the eighth day and death occurred on the fourteenth day after inoculation.

Either on account of greater susceptibility or due to the larger inoculating dose these animals showed more pronounced cholera lesions than the preceeding. In both there were marked splenic engorgement, hemorrhagic lymph nodes, red splotches on the skin and inflamed areas in stomach and colon. In the latter region also were extensive hemorrhages as also in points in the capsule of the kidneys. The lungs in each case showed small areas of red hepatization.

Culture tests in both these pigs yielded growths of *B. suipestifer* in purity. Microscopic examination showed these bacilli present in large numbers in the internal organs.

The bacillar invasion (*B. suipestifer*) in these cases was obviously not dependant upon external infection nor transmitted by the inoculation.

The pigs, 6 to 11 of list in Table I, were from the same farm. This was a subacute disease with prominent pneumonic lesions. Four of the six animals showed the bacillar infection.

Nos. 12 and 13 of table showed relatively few lesions and were free from infection.

Nos. 15 and 16, although they died from a disease of rapid course, showed no severe lesions post-mortem and yielded no growths.

Of Nos. 17 to 21 inclusive no ante-mortem history was obtained.

No 22 showed a condition occasionally found in dissecting hogs dead of cholera (and possibly occurring independently). The abdominal viscera, and especially the liver, even immediately post-mortem is rank smelling and porous being permeated with gas. Microscopic preparations show a spore containing fusiform bacillus which fails to grow in aerobic culture. The identity of this organism we have not yet worked out. It seems to be an ante-mortem infection.

Nos. 23 to 29 inclusive may be considered (so far as the original infection is concerned) as coming from one cholera outbreak since they were obtained on adjacent farms, infection spreading from one to the other. The disease originated among a lot of hogs bought up in the surrounding country for feeding purposes. Vaccination (serum alone) seemed to have little effect in curtailing the outbreak. The antiserum used was made by the use of stock yards virus.

It can be seen from Table I that organisms of the *B. suipestifer* type were

obtained in cultures from none of these. Nos. 23, 24, 25 were obtained from the first farm attacked and after the disease had prevailed for three weeks. The next four were among the first hogs affected on the adjacent farm. In these the disease might be considered subacute. Thoracic lesions were prominent in two and ulcerative intestinal lesions in all of them either slight or absent. No uniformity is seen in bacteriologic findings except the absence of *B. suipestifer*.

With this strain of virus characterized by the absence of *B. suipestifer* in the internal organs, a number of inoculation experiments were carried out in order to learn whether this feature would be perpetuated and to observe the effects and post-mortem lesions in the inoculated animals.

EXPERIMENT VI. One pig, weight 45 lbs., inoculated with 10cc of blood from No. 24 of Table 1, sickened in six days but survived for four weeks after inoculation.

Another receiving a similar dose of blood from No. 28 of Table I showed no sickness for two weeks. The disease followed a chronic course with death in seven weeks.

In both the intestinal ulceration, though not great, was the most prominent lesion. *B. Coli* was obtained from the organs of one, the other yielding no growth.

Seven other pigs of same weight similarly inoculated with smaller amounts of the same virus (2cc) showed sickness quite uniformly on the seventh or eighth day with fever and by the tenth day diarrhoea and wabbling gait. One of these died and the others were killed (to supply blood for immunizing purposes) all on the tenth day. The one that died had an extensive hemorrhage into the large intestine. The others showed few lesions and these chiefly intestinal (small ulcers and inflamed areas). Culture tests were negative in all.

The absence of bacillar (suipestifer) infection in all the animals studied in this outbreak and in the series of animals inoculated with this strain of virus may perhaps be explained by the absence of virulence, that is, natural attenuation of the virus. The disease although generally fatal was never acute and sometimes quite chronic in character.

Nos. 30, 31 and 32 are chiefly of interest on account of the prominence of the pulmonic lesions and absence of positive indications of cholera. They were from a bunch of ten pure bred shoats of around 60 lbs. weight, the history of which showed a possibility of exposure to cholera. They died after a short period of sickness (1 to 4 days) and on dissection showed similar pathologic changes. There was bilateral broncho-pneumonia involving the anterior two-thirds of lungs which were studded with yellow necrotic and purulent patches; also pleurisy with membranous adhesions. The mucous membrane of the cecum and colon was covered in most of its extent with pin head sized yellow points of exudate on top of the intestinal ridges while between these the membrane was of a deep red.

Culture tests from spleen and liver were negative. Lungs showed on microscopic preparations a mixture of species.

Such intestinal lesions are not typical of cholera and have in fact been described as characteristic of swine plague.

EXPERIMENT V. The following tests were made with this material:

1. A fifty pound pig was placed in the shed in which the above described animals (30 and 31 of Table I) had died and which had not been disinfected.

It was inoculated subcutaneously with blood and urine from pig No. 30. It was sick and refusing food on the ninth day and on the twelfth day when unable to stand was killed for dissection. It showed on examination, besides a local necrotic cavity, only a greatly enlarged spleen and hemorrhagic lymph nodes.

2. This pig of similar size to the above received in subcuta an injection of defibrinated hog cholera blood and along with this an emulsion scraped from the diseased lung of pig No. 31, the object being to learn if these pneumonic lesions would be reproduced when aided by a simultaneous cholera infection. It was kept in a disinfected shed.

This pig was sick on the sixth day with a temperature of 106° and appeared to get worse for several days but by the twelfth day was evidently improving. It was killed for dissection.

There was no evident pneumonia or pleurisy; petechiæ well marked on renal capsule; cardiac end of stomach covered by a grayish yellow exudate and a few small superficial ulcers in the large intestine.

Culture tests from the liver and spleen of both these experimental animals were negative.

The results showed that the pulmonic complication was not inoculable in either case. The disease induced by the inoculation in these cases was, if not typical, at least suggestive of cholera.

Nos. 34 to 37 inclusive. These were four out of a lot of seven shoats weighing about 60 lbs. each, which were infected when received at the serum plant pens. The symptoms and post mortem findings in these were quite typical of Cholera of a subacute character. As is shown in table the cultural tests in all were negative.

EXPERIMENT VI. The other three of this lot, (designated a, b and c) which showed no plain symptoms of infection at the time were inoculated with hog cholera virus as follows:

Pig *a*. With "Winslow virus" representing the type of disease prevailing in general in this State.

Pig *b* and *c* with stock yards virus (carbolized).*

Results. Pig *a*, which received 5cc of Winslow virus (blood injected into muscles) was refusing food on third day and was killed when very sick on sixth day. It showed engorged spleen, severe peritonitis and intestinal ulcers, obviously resulting from the original infection.

Culture tests negative.

Pig *b*, which received 2cc of stock yards virus was refusing food on the fourth day and grew worse with a temperature of 107° on the sixth day and profuse black diarrhoea. It was then killed for dissection. The post-mortem showed enlarged spleen and many dark based ulcers about the size of a dime in the cecum and colon. These were doubtless due to the original infection and not the inoculation.

Culture tests gave pure growths of *B. suipestifer.*

Pig *c* seemed to have immunity as it never showed sickness.

The interest in this experiment lies in the result of the culture tests in pig *b*, the mixed general infection with *B. suipestifer* being without doubt due to the inoculation and not to the original naturally acquired infection. The experiment does not show whether the infecting bacilli were derived from the

*Kansas City.

injection or of intestinal origin. It shows merely the difference between the two strains of cholera virus one uniformly unaccompanied with this bacillar complication and the other (as has been found by other tests) most commonly associated with it.

The remaining three cases in Table I were from two farms in adjoining counties where the hogs had been dying for some weeks. These also were negative to culture tests.

The outbreaks listed in Table 1 were selected on account of the opportunity they afforded for bacteriologic study. They represent closely enough the character of hog diseases which prevails in this State.

Other outbreaks of disease in hogs have been investigated which were considered by the owner or his neighbors to be something else than cholera on account of some supposed unusual symptoms such as prominence of cutaneous lesions (giving rise to the term "head disease"), slow course of the disease compared to what he had been accustomed to in the North, or the apparent failure of vaccination in controlling it. In every instance where there was a real enzootic disease, that is, one fatal to a considerable number of animals *the dissection of a few has brought to light the lesions of cholera such as* shown in the above table. This disease though quite commonly fatal in the end, is of a slow subacute or chronic type as a rule. It affects chiefly pigs and shoats under four months probably because the older stock has acquired immunity. The open range mode of hog raising which is common in this State with the scant feed and consequent slow growth may have an influence in diminishing susceptibility to cholera. It certainly does modify the course of an outbreak by indefinitely prolonging it. There is, however, evidence enough that the strain of hog cholera virus itself is subject to variation in virulence independent of complicating infections.

With the strain of virus referred to above as the "Winslow virus", as well as that obtained from the outbreaks represented by cases 23 to 29 in Table 1, many other inoculations have been made besides those reported in the experiments above described. There was an almost invariable response to these inoculations evident on the sixth to the eighth day by a reluctance at the feeding trough. The temperature would then be found from 105° to 107° as a rule. Diarrhoea was frequent but not constant. Eye symptoms were rare (conjunctivitis) but a catarrhal balanitis shown by pouching of the sheath was a frequent occurrence in males. A few animals would die rather suddenly within a day or two of first symptoms usually with large hemorrhages into the cecum or colon. In others which were allowed to live the disease would become chronic and of two to three weeks duration. The post-mortem lesions when killed within two weeks of inoculation were usually of slight extent and, whether in animals that were killed or those allowed to die naturally, culture tests were nearly always negative. In no instance were bacilli giving the evidence presumptive of the hog cholera group obtained from animals infected with these strains of virus. *Whether this was due to absence of these organisms* in the original virus or to some peculiarity (deficiency in virulence?) in the filterable virus itself is not quite clear but I am inclined at present to favor the latter theory.

Table II. LESIONS AND BACTERIOLOGY OF MATERIAL FROM SERUM PLANT.
(T, test pigs; H, hyperimmunes)

No.	Mode of Infection	Bacteria in Original	Bacterial Findings	Lesions
1	Inoculation	B. Suipestifer	B. Suipestifer	*Spleen*; l. nodes (submax. only).
2 (T)	S. S. Vac.	B. Suipestifer	B. Suipestifer	Kidneys; l. nodes (slight lesions).
3 (T)	Vac. and exposed		B. Suipestifer	*Spleen*; l. nodes; ulcers
4 (H)	S. S. Vac.		B. Suipestifer	Spleen; l. nodes; hemorrhages in colon.
5	Exposure		B. suisepticus	*Purulent pneumonia*; *pleurisy*; no other marked lesions.
6	Inoc.		Non-fermenting species from liver *only*	L. nodes; kidney; petechiæ in cecum and colon
7	Exposure		Negative	Spleen; l. nodes; infarcts in lung.
8	Inoc.		B. Suipestifer	L. nodes; cecal ulcers.
9	Exposure		Negative	Spleen (slight); no other lesions.
10	Exposure		Negative	No lesions; ante-mortem symptoms severe.
11	Exposure		Negative	Spleen; small ulcers and petechiae in cecum.
12	Exposure		Negative	No distinct lesions; ante-mortem symptoms only.
13	Inoc.		B. Suipestifer (liver)	Spleen; l. nodes (all slight).
14	Exposure		Negative	No plain lesions post-mortem.
15	Inoc.		B. *Coli*	Spleen; *kidneys*; l. nodes.
16	Exposure		Negative	Spleen; *cecal ulcers*; pneumonia.
17			Negative	Spleen; kidney (slight lesions).
18	Exposure		B. Suipestifer (spleen) (B. Coli, liver)	*Spleen*; *kidneys*; l. nodes (submax.).
19	Exposure		Negative	No plain lesions.
20	Inoc.		B. Suipestifer	Cecal ulcers.
21	Inoc.		Negative	*Cecal ulcers*; l. nodes (submax.).
22	Inoc.		Negative	Cecal ulcers; l. nodes (submax.).
23 (H)	Inoc. (large)		B. Suipestifer	*Spleen*; local lesion large.
24 (H)	Inoc. (large)	B. Suipestifer	B. Suipestifer	*Spleen*; liver; local lesion.
25 (T)	Vac. and exposure		Negative	*Ulcers*; l. nodes.
26	Inoc.		Negative	Spleen; l. nodes (submax.).
27	Inoc.	Negative	B. Suisepticus	*Kidneys*; l. nodes.

28	Inoc.	Negative	Negative	No lesions found.
29			Negative	Spleen; kidneys; l. nodes.
30			Negative	No lesions found.
31			Negative	Spleen ?; no other lesions.
32	Inoc.	Negative	B. Suisepticus	Spleen; pneumonia; pleurisy.
33	Inoc.		Negative	Spleen; kidneys; *cecal ulcers.*
34	Inoc.		B. Suipestifer (liver)	*L. nodes*; (mesenteric especially); cecal erosions.
35	Inoc.		B. Suisepticus	*Spleen*; cecal ulcers (superficial); hemorrhages
36	Inoc.		Cocci	*Spleen*; kidneys; cecal ulcers (small); l. nodes.
37	Inoc.		Negative	Spleen; l. nodes; cecal exudate in patches
38			Negative	No plain lesions.
39	Inoc.		Negative	Liver (parenchymatous degeneration); kidneys
40 (H)	Inoc. (large)		Negative	Spleen; lung infarcts.
41			Negative	Spleen; l. nodes (submax.); lesions slight.
42			Cocci (liver only)	Spleen; *kidneys*; l. nodes; hemorrhages in gut.
43			B. Suipestifer	L. nodes of mesocolon (slight lesions).
44 (T)	S. S. Vac.	B. Suisepticus *(Blood of No. 35)*	Negative	*Spleen*; no other lesions.
45	Inoc.	B. Suisepticus (Blood of No. 32)	Negative	Spleen; kidneys; cecal ulcer; l. nodes.
46	S. S. Vac. (intravenous)		B. Suipestifer	*Spleen*; *pneumonia*; ulcers.
47	Exposure		B. Suipestifer (impure Cult)	*Spleen*; *pneumonia*; (all of one lung); l. nodes.
48	Inoc.		B. suipestifer and cocci	*Spleen*; kidney; ulcers (slight); l. nodes.
49	Inoc.		*B. suipestifer and cocci*	Spleen; *l. nodes*; kidneys.
50	Inoc. (intravenous)		B. Suipestifer	Infarcts in lung; kidneys. Death in five days.
51	Inoc. (intravenous)		B. Suipestifer	Spleen; l. nodes; ulcers (slight).
52	Inoc.		B. Suipestifer	Spleen; kidneys; l. nodes (mesocolic).
53	Inoc.	Negative	B. Suipestifer	Kidneys; cecal ulcers (slight lesions).
54 (T)	S. S. Vac.	B. Suipestifer	B. Suipestifer	No plain lesions; ante-mortem symptoms severe.
55 (T)	Exposure		B. Suipestifer	*Spleen*; liver (perihepatitis); pleurisy; *ulcers.*
56 (T)	Exposure		Negative	Kidneys; l. nodes; ulcers.
57 (T)	S. S. Vac.		B. Suipestifer	Spleen; l. nodes.
58	Inoc.		B. Coli (liver only)	L. nodes (submax.); spleen; cecal hemorrhages
59	Inoc.	Negative	Cocci and non-fermenting bacilli	Ulcers only (chronic case).

Table II. LESIONS AND BACTERIOLOGY OF MATERIAL FROM SERUM PLANT.—(Continued)
(*T*—*test pigs*; *H*—*hyperimmunes*)

No.	Mode of Infection	Bacteria in Original	Bacterial Findings	Lesions
60	Inoc.		Negative	Kidneys; l. nodes; hemorrhages in colon.
61	Inoc.	Negative	Negative	Kidney; ulcers (slight lesions).
62	Inoc.	Negative	Negative	No obvious lesions post-mortem.
63	Inoc.	Negative	*Filamentous* bacilli (liver)	Large intestinal hemorrhage only.
64	Inoc.	Negative	Negative	Kidneys; ulcers; l. nodes.
65	Inoc.	Negative	Negative	A few petechiae on lungs and kidneys.
66	Inoc.	Negative	Negative	Small ulcers (lesion slight).
67	Inoc.	Negative	Negative	L. nodes; spleen; ulcers.
68	Inoc.	Negative	Negative	Spleen; l. nodes (submax.).
69	Inoc.	Negative	Negative	No plain lesions (severe ante-mortem symptoms).
70	Inoc.	Negative	Negative	Spleen; l. nodes (*submax.*).

Table No. 2 contains the record of results obtained from examination and bacteriologic tests on material obtained from the department having in hand the making of antiserum. The data as to the source of infection is only available here in a few cases, consequently the third column of the table is very incomplete.

It is shown in this table that of 70 cases examined *B. suipestifer* was found generalized in 21, of which 16 had been infected by inoculation (cholera infection), 4 by exposure and one without record. Of the animals furnishing the blood for inoculation bacteriologic data is on record for 21. It is seen that in the four cases in which *B. suipestifer* was present in the animal furnishing the virus for inoculation this organism appeared also in the inoculated animal as a mixed infection. It occurred four times in animals infected by exposure only and in all cases (3) which were recorded as having received an intravenous inoculation. Also in five of the six hogs which died after serum-simultaneous vaccination. As for the post-mortem lesions present in animals showing this mixed infection it will be seen that there is no uniformity, that is none which occur only in such cases. Two are recorded as showing only slight lesions.

Bacillus suisepticus was obtained in one case in which there were no pulmonary lesions reported. In the two cases in which this species was present in the original virus it was not found in the inoculated animal, nor were pulmonary lesions present.

Experimental mixed infection. To obtain more direct evidence of the effect of mixed infection with *B. suipestifer* and hog cholera virus (filterable virus) experiments were carried through in which pigs were infected simultaneously with cholera virus (blood) and with cultures of the bacilli, in comparison with others receiving the blood infection alone.

The virus employed was that already referred to as the "Winslow virus" which had never been found to give rise to this mixed infection in previous tests. The culture of *B. suipestifer* employed was one which had been grown in the laboratory for 18 months. At the time it was isolated this strain was of less than the usual degree of virulence for guinea pigs. It had not previously been tested on pigs

EXPERIMENT VII. The plan as carried out was to inoculate two pigs with cholera blood alone, two with blood and swine pest bacilli cultures and two with blood along with a simultaneous feeding of these cultures. This last pen is called for in the experiment because bacterial infection in cholera, if transmitted at all, presumably occurs by ingestion. Moreover, infection experiments on hogs with B. suipestifer alone is more frequently successful by this mode than by subcutaneous inoculation. At the end of 7 days when all the experimental pigs were showing sickness additional pigs were added to each pen to learn the results of infection by exposure.

The details of this experiment are shown in Table III:

Table III. EXPERIMENTAL MIXED INFECTION WITH HOG CHOLERA AND B. SUIPESTIFER.

	No.	Treatment	Sick on	Killed on:	Bacterial findings	Lesions
Pen. III	1	Inoc. 5cc. blood	7th day	10th day	Negative	*L. nodes*; kidneys; spleen, ?; (Temp. 106)
	2	Inoc. 5cc. blood	8th day	10th day	Negative	Skin; l. nodes; *spleen*; kidneys. (Temp. 105)
	3	Put in pen on 7th day	7th day	14th day	Negative	Skin; *l. nodes*; spleen; kidneys
	4	Put in pen on 7th day	7th day	21st day (died)	Negative	L. nodes (slight); exudate and hemorrhages in cecum and colon
Pen. IV	5	Inoc. 5cc. blood and 20cc. culture	3rd day	9th day	B. suipestifer	L. nodes (jaw); *spleen*; *kidneys*;
	6	Inoc. mixture of 5cc blood and 20cc. cul.	3rd day	9th day	B. suipestifer	Skin; l. nodes; *spleen*; *kidneys*; cecal exudate
	7	Put in pen 7th day	? 20th day	Survived		
Pen. V	8	Inoc. 5cc. blood and fed 30cc. culture	3rd day	8th day	B. suipestifer	*Kidneys*; *exudate in cecum and colon*
	9	Inoc. 5cc. blood and fed 30cc. culture	3rd day	9th day	B. suipestifer	L. nodes; spleen; kidneys; exudate on mucosa of cecum and colon
	10	Put in pen 7th. day	7th day	24th day (died)	Negative as to B. S.	*Spleen*; no other plain lesions

The results of this experiment, shown in brief in the table, require further explanation especially in reference to the antemortem symptoms and pathologic changes found post mortem.

The pigs in pen III, inoculated with blood only, showed the usual period of incubation, six to eight days. Food was not entirely refused and weakness of gait was not noticable. Temperature on the ninth day was 104.6° and 106.4°. There was no diarrhoea and both would probably have lived for several days at least. They were killed on tenth day in order to obtain a more exact comparison with those of the other pens. Pig No. 1 showed no discoloration of the skin while in No. 2 this was a marked feature. A large wine colored patch ineffaceable by pressure was present on the scrotum and red wheals all over the venter surface and inside of thighs. The large intestine in both these pigs was free from any obvious lesions.

In pen IV, the pigs (which had received the inoculation with blood and culture) were both refusing food on the third day and on the fifth were extremely sick and wabbling in gait. On the seventh day pig No. 5 showed dyspnoea which increased until it was killed on the ninth day. No 6 did not exhibit this symptom but became so weak by the ninth day, when it also was killed, that it was unable to stand and would probably have died within a few hours.

Post mortem. No. 5. No cutaneous lesions, lymph nodes large but only slightly congested; spleen large, dark and soft; lungs uncollapsed and highly edematous; kidneys studded with punctiform hemorrhages. In large intestine no changes except a very few small inflammatory nodules suggesting commencing ulcers.

No. 6. Large wine colored areas on belly, and on inner aspect of thighs elevated red wheals. Inguinal lymph nodes large and hemorrhagic. Minute hemorrhages on lung and kidney. Spleen engorged. Mucosa of cecum and colon shows in addition to the small nodular lesions seen in No. 5 a number of isolated patches of superficial yellow adherent exudate.

Pen V. In this pen the two pigs (in addition to the blood inoculation) were infected with *B. suipestifer* by feeding 30 cc of a 24 hour broth culture to each. It was given in milk after twelve hours fasting. These pigs were affected very much like those of Pen IV, refusing food on the third day and becoming progressively worse. No. 8, by the seventh day was unable to stand. It was killed on the eighth day and No. 9 on the ninth, both being apparently in a dying condition.

Post mortem. No. 8. Eyes gummy; slight pouching of sheath; lymph nodes large but not hemorrhagic; kidneys pale with numerous petechiæ visible on surface and on section. Cecum and part of colon adjacent has an ochre yellow exudate on mucous membrane not easily removed. No evident ulcers. No. 9 has hemorrhagic lymph nodes and engorged spleen, kidneys only slightly specked otherwise similar to No. 8.

As for the bacteriologic tests all four animals artificially infected with *B. suipestifer* and hog cholera blood yielded pure cultures of this species from liver and spleen and the microscopic examination showed these to be abundantly present.

The exposure tests. As shown in the table two pigs were introduced into Pen III and one each into Pens IV and V after the pigs already in these pens had become obviously sick. They were exposed to infection by close

cohabitation for several days and later when the original pigs were removed, by exposure in the uncleaned pens. The resulting infection must have taken place at once since sickness appeared after the usual incubatory period which follows inoculation, that is, seven days. The pens were really small 6 by 10 feet wooden floored sheds which compelled the closest kind of association.

In Pen III one pig (No. 3.) was killed when very sick after two weeks exposure, the other, though also quite sick, was allowed to live while it would and it survived a week longer. The only points calling for notice further than what is supplied by Table III are that No. 3 presented no intestinal lesions while in No. 4 the condition was very similar to that found in No. 8 and 9 (infected with *B. suipestifer*) but the exudate was more closely adherent and when scraped off left more marked erosions.

In Pen IV the exposure pig was never plainly sick although said to have fed poorly about the 20th day of exposure. It was later injected with large amounts of cholera blood and proved to be immune.

Pig No. 10, exposed in Pen V, developed a chronic case of cholera from which it died in 24 days from first exposure. It showed few pathologic changes on dissection. The spleen was large with soft red pulp. No intestinal changes.

All three of these exposure pigs proved negative to culture tests.

To summarize the information gained by this experiment we would notice in the mixed infection:

1. The shortening of the incubatory period.

2. The greater severity of the symptoms and more rapid course of the disease.

3. The absence of any essential and uniform differences in the pathoanatomical changes as seen on dissection. The peculiar form of exudative inflammation with necrosis of the surface epithelium of the large intestine which occurred in both of the pigs in Pen V (infected with bacilli by feeding) and in one of Pen IV (infected by inoculation) was present also in the exposure pig of Pen III which survived for three weeks. Since this condition quite regularly results from successful infection experiments by ingestion of cultures of *B. suipestifer* alone we must look upon it as an inflammatory reaction due to the presence of this bacillus.

In pig No. 6 the bacilli occasioning this reaction, if derived at all from those inoculated, must have reached the intestine by way of the blood. The occurrence of a similar condition in pig No. 4 can only be explained by assuming that it was due in this case to bacilli already present in the intestine. Absence of *B. suipestifer* from the internal organs would imply that the action here was a purely local one dependant in some way on a prior or associated injury of the mucosa due to the filterable hog cholera virus. When the number of virulent bacilli of this species is excessive as in experiments in feeding cultures alone, the same condition can apparently be brought about without this accessory cause.

4. The absence of any evidence of transference of the bacillar infection to the exposed animals in Pens IV and V should also be noticed. The infection in these was really less active apparently than in Pen III where no bacterial cultures had been used.

This negative results tends to show that *B. suipestifer* infection in hog

cholera is probably not dependent to any great extent on transference from animal to animal. However, any positive assertions of general application on this question must be based on more extensive tests carried out under varying conditions especially as to degree of virulence both of the filterable virus of cholera and of the strain of bacilli employed. It is known that the artificially induced bacillar disease alone does not exhibit the contagious properties of true hog cholera but under favorable conditions is occasionally transmitted by cohabitation. *

5.' Still another point to be noticed is the certainty with which general infection with *B. suipestifer* results when this germ is artificially introduced (by inoculation or ingestion) in conjunction with the true hog cholera virus. Reference to Tables 1 and 2 will supply evidence corroborative of this fact. In contrast to this is the known fact that infection experiments with the bacillus alone are often negative of result.

Immunity to bacillar infection. Since infection with *B. suipestifer* is not known to occur unassociated with true hog cholera infection, except when artificially induced, a complete immunity to the latter virus should indirectly afford protection against the bacillar invasion as well. Moreover, most samples of hog cholera antiserum contain antibodies against this organism as indicated by their high agglutinating properties, a reaction, presumably, to the bacilli of this species contained in the blood with which the animal was hyperimmunized. How far this bacillar anti-body content in serum may aid in increaseing its potency in preventing hog cholera as it occurs on the farms is a question which has not yet been investigated. Probably in "serum alone" vaccination its importance is slight since in the naturally acquired disease bacillar infection may succeed but does not precede the filterable virus infection. In "serum simultaneous" vaccination, when the virulent blood injected contains bacilli of this species, we should expect, on theoretical grounds at least, that bacillar anti-bodies should be present in the antiserum, otherwise the animal thus vaccinated would be in the same condition as one inoculated with bacilli alone. In the practice of "serum simultaneous vaccination" accidents arising from this cause are no doubt of occasional occurrence. That they are not frequent may be explained by the facts: (1) that most samples of antiserum contain bacterial antibodies; (2) most samples of virus do not contain bacilli; (3) inoculation of bacillar cultures subcutaneously are often ineffective.

Serum simultaneous vaccination after immunity is established would in general imply immunity against bacillar infection as well. This may be produced in either or all of three ways: (1) by reason of the bacillar anti bodies in the serum; (2) by multiplication in the body of the animal of bacilli introduced with the virus (though short of producing obvious sickness); (3) by a multiplication, either general or local only, of bacilli having their origin within the intestine (such as occurs after inoculation of bacteria-free virus).

Agglutinins to *B. suipestifer* in hog cholera antiserum we assume to be due mainly to a reaction against the bacilli injected with the blood used in hyperimmunizing or to the invasion of bacilli from the intestine when these are absent from the injected blood. When the bacilli are absent from

*Bul. No. 105, Ark. Exper. Station.

the blood and the strain of virus used is such as does not bring about this generalized mixed infection, the antiserum will contain few or no bacterial antibodies.

In our experiments a considerable amount of antiserum was made by the use of blood from hogs infected with the strain of virus mentioned above which was never found associated with *B. suipestifer*. This serum from a number of different hogs was found to be relatively low and sometimes almost devoid of agglutinating properties but it was fully up to the standard in preventing disease when used against the strain of virus with which it was made,—20cc protecting against an inoculation of 2cc of blood.

In order to learn if pigs thus vaccinated were immune to bacillar infection or to strains of virus usually occasioning mixed infection the following experiments were carried out:

EXPERIMENT VIII. Three pigs which had withstood such a test of serum virus vaccination, along with three previously untreated pigs as controls, were used in the test. One was inoculated with the same strain of virus previously used on it and one with stock yards virus. These two received also an inoculation of a culture of bacilli on the opposite limb. The third was inoculated with stock yards virus only. Two control animals received similar double inoculations while the other received an inoculation of stock yards virus only. The plan of this experiment is shown in Table IV.

The three test pigs had, six weeks previously, been S. S. vaccinated in testing serum, receiving 10, 15, and 20 cc of serum and 1.5cc each of defibrinated blood , which test they successfully withstood. At the time of this second test they weighed from 50 to 60 lbs. each and were in good condition. A suspension of one agar slant culture was divided among the four pigs, two tests and two controls, this being approximately equivalent to one cubic centimeter each of a 24 hour broth culture. The dose given was purposely much smaller than in the previous test (Table III) which seemed to be unnecessarily large.

Table IV. TEST OF IMMUNITY OF S. S. VACCINATED PIGS TO BACILLAR INFECTION.

	No	Previous Treatment	Inoculations	Sick	Killed	Bacterial findings	Lesions
	1	S. S. vaccinated	5cc. blood (on right) 1cc. cult. (on left)	----------	----------	----------	----------
Pen. V	2	Untreated	Same as No. 1	4th day	14th day	B. suipestifer	*Spleen*; l. nodes; *ulcers*; stomach
	3	S. S. vaccinated	3cc. blood (stockyards) and 1 c c. culture	----------	----------	----------	----------
Pen. VI	4	Untreated	Same as No. 3	5th day	19th day	B. suipestifer	*Spleen*; pneumonia; pleurisy; *ulcers*
	5	S. S. vaccinated	5cc. blood (stockyards)	----------	----------	----------	----------
Pen. VII	6	Untreated	Same as No. 5	6th day	7th day	B. Coli *	Peritonitis; pleurisy; l. nodes (mesocolic)

*B. suipestifer may also have been present, its fermentative action being masked by that of the colon bacillus.

It is seen that the three previously vaccinated pigs were resistant to this inoculation test, the immunity resulting from the earlier vaccination with an antiserum low in agglutinating properties, being sufficient to protect against the later inoculation of both strains of virus conjoined with cultures.

We infer from the result of this experiment that aggluination to *B. suipestifer* is not an essential to hog cholera antiserum in serum simultaneous vaccination provided the same strain of virus is used in vaccination as was employed in producing the serum.

However, the immunity thus acquired, while apparently sufficient to protect against subcutaneous innoculation of small doses of bacilli, seemed to break down under intravenous inoculation of large amounts of bacilli containing blood. Pigs No. 1 and 3 of the above experiment were each one month later, injected in the vein of the ear with 400 cc of defibrinated blood coming from a hog which, having been inoculated with stock yards virus, had developed a mixed (*suipestifer*) infection.

Both sickened on the following day. The sickness of one was temporary only and it was apparently well within a week. The other died after 11 days increasing sickness. It showed on post mortem typical lesions of cholera and yielded cultures of *B. suipestifer*, this organism being also found abundantly in microscopic preparations from spleen and liver.

It may be mentioned here that most serum hogs which die during treatment (when not due to mechanical overloading of the circulation) show a generalized *suipestifer* infection which is suggestive of insufficient preliminary immunization against this bacillus.

OTHER ASSOCIATED BACTERIA. In this study of the bacteria associated with the filterable cholera virus in producing mixed infection, most attention has been given to those which conform to the type of the hog cholera bacillus the presumptive evidence of which in culture tests is the fermentation with gas evolution of dextrose and absence of gas in lactose broth fermentation tubes. All cultures giving this preliminary reaction were assumed to contain this species either in pure or mixed culture. To verify the correctness of this assumption many cultures were subjected to further biologic tests and found in every instance to give all the cultural characters of this type.

Next to *B. suipestifer* the most frequent organism obtained in culture tests from cholera diseased hogs is some variety of the colon group. None of these have been closely studied or experimented with in this investigation. The colon bacillus is a very frequent contamination in culture tubes due to defective technique. The average laboratory assistant can be depended on to obtain growths of this germ from any specimen. It is one of the earliest post mortem invaders of the abdominal viscera and probably of less frequent occurrence as an ante-mortem infection than our culture tests indicate. In my experience this species has not been found with sufficient frequency in any one outbreak to justify the view that this form of mixed infection may be characteristic of certain outbreaks, suipestifer infection of others. To admit this would almost imply that these mixed infections were due to contagion of which there is no sufficient evidence.

One undetermined species of coccus morphologically resembling *M. catarrhalis* has been found in several cases in small numbers in the spleen, liver and blood in hog cholera. It grows rather feebly on ordinary media and cultures are non-pathogenic for guinea pigs

B. pyocyaneus occurs occasionally in the pneumonic lesions of hog cholera and more frequently in large local lesions following inoculation. These and a variety of other bacterial organisms which may occasionally appear in the culture tubes (see Table II) in isolated cases, can not be considered as of pathologic significance except in showing the tendency to secondary infections which exist in this disease.

B. suisepticus (see Table II) has been found infrequently during this investigation. In all except one case it was associated with marked pulmonary lesions—pneumonia and pleurisy. As has been already described, these were shown by experiment to be secondary to hog cholera infection and no plain evidence of transference of this complication by contagion was supplied by these cases. It is known that different strains of this species exhibit extreme variation in virulence and it is not improbable that a highly virulent strain may be capable of spreading by contagion this pulmonary complication so as to make it characteristic of a hog cholera outbreak. The frequency of pectoral lesions in certain outbreaks of cholera seems to favor this view.

From reports that we have received it seems also that vaccination is less effective in combatting outbreaks of this kind, but the available data on this subject is scanty where it ought to be plentiful.

A closer study of hog diseases as they occur on the farm and more careful observation of the effects of vaccination in outbreaks showing different types of lesions is needed in order to place serum vaccination on a more rational basis.

Varieties of hog cholera-like bacilli. Of the many cultures obtained from the organs of cholera diseased hogs giving the fermentation reaction which I have regarded as presumptive evidence of *B suipestifer*, a number were selected for closer study, especially in regard to their fermentative action on various carbohydrates. Table V gives the results obtained with seven strains coming from as many different animals. Three of these were obtained from different outbreaks of cholera and the remaining four from virus pigs inoculated with different samples of virus. All gave the general cultural reactions ascribed to the hog cholera or *B. enteritidis* subdivision of the colon-typhoid group of bacilli.

The one feature in which these strains showed a considerable variation was in the production of indol in Dunham's peptone solution after one week's incubation. According to the text books indol production in peptone solution is not a feature belonging to this species although it may occasionally occur. As may be seen in the subjoined table in which two separate tests are recorded, this reaction was obtained to an appreciable degreee in six out of the seven strains tested. The reaction in all was slight in comparison to that obtained from colon bacilli cultures.

Table V. Indol reaction in growths of *B. suipestifer* in peptone solution.*

Strain	Test of 1-14, '13	Tests of 5-6, '13
I	Distinct	Distinct
II	Trace	Slight
III	Distinct	Distinct
IV	Distinct	Slight
V	Distinct	Distinct
VI	Negative	Negative
VII	Distinct	Distinct

*Tests by laboratory assistant C. S. Davis.

In the comparative fermentation tests one per cent solution of the various carbohydrates was contained in nutrient broth made from meat extract and the solution sterilized by discontinuous steaming in the Arnold. In control tubes of this medium without the addition of sugar no gas formation occurred under growth of any of the strains tested. Hence for the purpose of this test a preliminary growth of B. Coli in the culture media was considered unnecessary. The media was made neutral to litmus and the final reaction was determined by litmus test paper only, no titrations being made. Tubes were used of 10 cm length of arm and 15cc capacity when properly filled. All cultures were first grown for 24 hours in nutrient broth and from this, one platinum loop transferred to the fermentation tubes. These were incubated at 37° and daily records made until gas evolution ceased.

Duplicate tests were made at an interval of several weeks.

Table VI. FERMENTATIVE ACTION OF B. SUIPESTIFER*

One Per Cent Solution DEXTROSE
(Tests Made Dec. 6, 1912; Mar. 6, 1913)
Gas Per Cent Developed, in Hours:

Strain Hours	24	48	72	Max	Reached in:	CO_2: H	Reaction
I	22	30	35	35	72	1:2.5	Acid
I	40	43	40	43	48	1:2.6	Acid
II	35	47	47	49	96	1:2	Acid
II	37	43	44	45	96	1:2.4	Acid
III	30	35	35	35	48	1:2	Acid
III	37	43	44	45	96	1:2.1	Acid
IV	28	40	40	40	48	1:2.6	Acid
IV	33	41	43	44	96	1:2	Acid
V	20	33	37	37	72	1:2.2	Acid
V	37	42	43	43	72	1:2.1	Acid
VI	20	38	40	40	72	1:2.6	Acid
VI	29	37	37	39	96	1:2	Acid
VII	37	52	52	52	48	1:2.9	Acid
VII	32	40	42	44	96	1:1.9	Acid

GALACTOSE

(Tests made Mar. 14 and May 6, 1913)

I	28	34	36	37	120	1:2.3	Acid
I	28	35	37	37	72	1:2	Acid
II	22	27	32	35	120	1:2.1	Acid
II	23	30	34	35	96	1:2.1	Acid
III	25	32	34	35	120	1:2.5	Acid
III	30	36	37	37	72	1:2.3	Acid
IV	25	29	32	34	144	1:2.3	Acid
IV	22	30	30	31	96	1:2.8	Acid
V	25	27	30	30	72	1:2.3	Acid
V	22	27	30	30	72	1:2.5	Acid
VI	22	30	30	30	96	1:2.5	Acid
VI	27	32	35	35	72	1:2.5	Acid

*Tests by C. G. Davis.

MALTOSE

(Tests Made Dec. 24, 1912 and Feb. 12, 1913)

Per Cent Gas Developed, in Hours:

Strain	24	48	72	Max	Attained in	CO_2: H	Reaction
I	10	30	30	30	48 hours	1:2.7	Acid
I	35	46	47	47	72 hours	1:1.9	Acid
II	10	30	30	30	48 hours	1:2.3	Acid
II	22	37	39	40	96 hours	1:2	Acid
III	10	30	30	30	48 hours	1:2.3	Acid
III	28	27	37	37	48 hours	1:2.6	Acid
IV	10	25	28	28	72 hours	1:2.5	Acid
IV	30	42	41	42	48 hours	1:2.3	Acid
V	10	30	32	32	72 hours	1:2.2	Acid
V	25	40	44	45	96 hours	1:1.8	Acid
VI	10	30	32	32	72 hours	1:2.5	Acid
VI	19	32	35	36	96 hours	1:2.6	Acid
VII	5	30	32	32	72 hours	1:2.2	Acid
VII	21	42	43	44	96 hours	1:	Acid

MANNITE

(Tests made Jan. 9; Feb. 12; Mar. 6, 1913)

Strain	24	48	72	Max	Attained in	CO_2: H	Reaction
I	45	50	50	50	48 hours	1:1.8	Acid
I	27	38	38	38	72 hours	1:2.5	Acid
I	35	42	44	45	96 hours	1:1.8	Acid
II	40	48	43	48	48 hours	1:2.3	Acid
II	25	35	38	38	72 hours	1:2.7	Acid
II	25	42	42	42	48 hours	1:2	Acid
IV	38	43	42	42	48 hours	1:2.5	Acid
IV	32	47	48	48	72 hours	1:2.1	Acid
IV	28	40	41	42	96 hours	1:2	Acid
V	40	51	48	51	48 hours	1:2.2	Acid
V	28	45	46	46	72 hours	1:3	Acid
V	43	56	56	56	48 hours	1:2	Acid
VI	43	55	52	55	48 hours	1:2.2	Acid
VI	18	42	47	47	72 hours	1:2.7	Acid
VI	39	49	48	49	48 hours	1:2.3	Acid
VII	35	40	38	40	48 hours	1:3.7	Acid
VII	20	42	45	45	72 hours	1:3	Acid
VII	34	44	45	45	72 hours	1:2	Acid
III	40	40	35	40	48 hours	1:3.7	Acid
III	32	45	45	45	48 hours	1:2.6	Acid
III	30	37	37	40	72 hours	1:2	Acid

It will be seen that the fermentative property of all these cultures are practically alike, variations in volume of gas between different strains being no greater than between different tests of the same strain.

Agglutination tests. These were made with living 24 hour meat broth cultures diluted with an equal volume physiologic salt solution. This formed the suspension of bacilli which was mixed with serum in amounts diminishing in series by one half so that each tube contained half the amount of serum of the tube preceeding it. The volume of suspension was 1cc and of serum dilution 1cc.

Method of procedure: Tube 1 received 1.9cc salt solution; all the others 1cc salt solution. To tube 1 was added 0.1cc serum. After mixing 1cc was removed from tube 1 to tube 2 and mixed. From this 1cc removed to tube 3 and this continued till the last tube was reached which re-

ceived no serum being used as a control. All the tubes then received 1cc of the bacillar suspension. Each tube therefore contained 2cc of fluid. In recording results of agglutination tests we have followed the practice commonly employed reckoning the dilution as the ratio of the serum to the total fluid.

The *same sample of antiserum, one of moderate agglutinating power*, was used for all. Tubes were incubated two hours then kept in the ice box over night. In the table the sign + means a complete reaction (sedimentation and clearing): + — indicates incomplete clearing. These are *the end reactions*

Table VII. AGGLUTINATION TESTS WITH ANTISERUM*

STRAIN	1-40	1-80	1-160	1-320	1-640	1-1280	1-2560	1-5100	1-10200	1-20000	1-40000
I	+	+	+	+	+	+	+	+—	+—	O	O
II	+	+	+	+	+	+	+	+—	+—	+—	O
III	+	+	+	+	+	+	+	+—	O	O	O
IV	+	+	+	+	+	+	+	+—	+—	O	O
V	+	+	+	+	+	+	+	+	+—	O	O
VI	+	+	+	+	+	+	+	+	+—	O	O
VII	+	+	+	+	+	+	+	+—	+—	O	O

The view which some have entertained that the bacilli which have been so generally cultivated from the organs of cholera diseased hogs and described as *B. suipestifer* or *B. cholerasuis* really include a variety of organisms presenting on closer study differences in cultural characteristics is not supported by this investigation where all the strains studied are *practically identical in cultural and biologic characters*.

All these strains were only feebly virulent for the guinea pig. In one-tenth cubic centimeter dose (subcutaneous) two cultures caused death in 10 to 14 days, respectively, two temporary sickness and three produced no obvious effect.

It seems therefore that cultures from the organs of cholera diseased hogs giving the fermentation tube reaction which we have called presumptive evidence of *B. suipestifer* are probably always this species. It is of interest that this same fermentation tube reaction has occasionally been found in city water tests here but never further investigated.

B. paratyphoid B of which a stock culture is kept in the laboratory was recently tested out on hog cholera antiserum in comparison with a strain of *B. suipestifer*. The result is here shown:

B. suipesifer (Strain III) agglutinated at dilution 1 to 10,000.

Paratyphoid B. agglutinated at dilution, 1 to 320. These figures are obtained *by the method of computing dilutions* described for Table VII. The actual ratio of serum to the undiluted 24 hour broth culture in each case is 1 to 2560 and 1 to 40.

REVIEW. A review of Tables I and II shows the infrequency with which bacilli of the hog cholera type occurs in natural outbreaks of the disease as compared with those artificially infected. The disease which

*Tests by C. G. Davis.

now mostly prevails in this State is *not accompanied by this mixed infection* either in animals naturally infected or in those inoculated from them. This can not be ascribed to a localization of the lesions in organs other than the intestine. It is probably, although the evidence is not quite clear on this point, due to deficiency in virulence a somewhat virulent type of disease being necessary in order to beget an invasion of the system by these bacilli. Inoculation (or feeding) of bacilli in conjunction with hog cholera virus of any strain, quite uniformly begets this mixed infection, but there is no evidence that suipestifer mixed infection in farm outbreaks results from contagion (transference of the bacilli from animal to animal). From Experiment VII it is clear that a generalization of B. suipestifer in the body of the animal greatly increases the severity of the disease and aggravates the ante mortem symptoms although producing no characteristic *post mortem lesions. However, this mixed infection* and increased virulence was not transmitted by cohabitation.

Certain strains of cholera virus, even when the bacilli are absent from the blood seem to, quite generally, beget bacillar infection by inoculation The same effect is produced by exposure infection with these strains. Hogs which are protected against true hog cholera virus by vaccination are also protected against infection with *B. suipestifer* (Exp. VIII) even when bacillar antibodies are lacking in the serum used in vaccinating. This immunity is insufficient to protect against large doses of bacilli given intravenously, hence serum hogs should be previously immunized by the use of serum showing a high agglutinating property and by virus which begets mixed infection. However, antiserum of low agglutinating power (for *B. suipestifer*) may be effective if used alone or in conjunction with the same strain of virus employed in producing it.

Although not directly an index of its potency against the true hog cholera virus, a high agglutinating power in a sample of antiserum (against *B. suipestifer*) would in general be an indication that it was produced by the use *of a virulent strain of cholera virus. This on the theory* that bacillar infection in hog cholera is chiefly dependant on the virulence of the disease.

Swine plague as an independent disease has not been met with during this investigation. In several instances in which the anatomical lesions were suggestive of this disease inoculation experiments have shown the primary infection to be that of cholera. (Exp. I, II, V.) The transference by contagion of the thoracic lesions which have been associated with swine plague (generally due to *B. suisepticus*) is a question which needs further investigation.